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Pullulan From Agricultural Waste Using *Aureobasidium pullulans* and Its Applications: A Review

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ABSTRACT

Pullulan is an extracellular homopolysaccharide produced commercially by the yeast-like fungus *Aureobasidium pullulans*. Owing to its unique physicochemical properties, such as film-forming ability, biodegradability, and non-toxic nature, pullulan has attracted significant interest for applications across diverse industries, including cosmetics, pharmaceuticals, food packaging, and agriculture. In recent years, the demand for sustainably derived biopolymers has intensified, prompting exploration of industrial waste and renewable substrates as cost-effective feedstocks for pullulan production. Despite its promising versatility, large-scale commercialization remains constrained by high production costs, low yield, and limited substrate flexibility. This review critically examines the potential of microbial-based pullulan production, highlighting both technological opportunities and persistent challenges. Particular emphasis is placed on the use of alternative substrates, strain improvement strategies, and bioprocess optimization to enhance yield and reduce costs. Advances in metabolic engineering, fermentation technology, and integrated biorefinery approaches are discussed as pathways to overcome current limitations. By synthesizing recent progress and identifying research gaps, this study aims to support future innovation in sustainable pullulan production and broaden its industrial applicability. Ultimately, optimizing production parameters and diversifying microbial systems may provide a viable solution to transform pullulan into a widely accessible biopolymer for global markets.

1. INTRODUCTION

1.1. Carbohydrates

The term "carbohydrate" encompasses a group of molecules including starch, sugars, and cellulose (Avenas 2012). Microbes produce a wide variety of carbohydrate-based polymers, such as bacterial cellulose, xanthan, levan, dextran, gellan, pullulan, curdlan, and scleroglucan. Pullulan is a linear homopolysaccharide that forms the backbone of maltotriose units (Nasrollahzadeh et al. 2021). Polysaccharides are hydrocolloids, water-attracting polymers, that find applications across the food, pharmaceutical, and cosmetic industries due to their unique physical and chemical properties.

In food, they serve as stabilizers, thickeners, film-forming agents, and emulsifiers in products like sauces, beverages, and bread (Pirsa & Hafezi 2023). In pharmaceuticals, they are used to produce hydrogel tablets, hard capsules, and matrices. Since many polysaccharide gels originate from plant and animal sources, researchers are exploring alternatives due to their limited availability for human consumption and non-renewability (Kostag & El Seoud 2021).

1.2. History

In 1958, Bernier first isolated the carbohydrate pullulan from *Aureobasidium* *pullulans*. A year later, Bender et al. conducted a structural analysis of a novel polysaccharide and named it pullulan. The basic composition of pullulan was elucidated during the 1960s (Abhilash & Thoma 2017).

1.3. Pullulan

Pullulan is a linear, biodegradable, extracellular, water-soluble homopolysaccharide produced by the yeast-like fungus Aureobasidium pullulans (Singh et al. 2019). It forms an amorphous, slimy mass on the outer surfaces of the fungus. Structurally, pullulan consists of repeating maltotriose units connected via α-(1,6) glycosidic bonds (Li et al. 2015). Its unique structure confers high water solubility and biodegradability, making pullulan a valuable and versatile biomaterial with significant application potential (Ates 2015). Pullulanase, a well-known debranching enzyme, plays a crucial role in breaking down pullulan. Hydrolysis of pullulan by pullulanase yields various oligosaccharides, including maltose, maltotriose, maltotetrose, isomaltose, and panose (Rajeeva et al. 2010). Maltotriose serves as the primary repeating unit within pullulan. Chemical modifications can be employed to enhance its physical properties.

Pullulan is tasteless, odorless, edible, non-toxic, non-mutagenic, and non-carcinogenic. It finds extensive use across multiple industries such as pharmaceuticals, food, cosmetics, biomedical fields, targeted drug delivery systems, waste bioremediation, and medical applications (Sugumaran et al. 2017).

1.4. Structure of Pullulan

Pullulan has the chemical formula $(C_6H_{12}O_5)n$, and its structure is composed of repeating maltotriose units, in which three glucose molecules are linked by α-(1→4) and α-(1→6) glycosidic bonds. Spectral data verify the presence of both α-(1→4) and α-(1→6) linkages in pullulan. Bernier was the first scientist to confirm that pullulan contains glucopyranose (Bernier 1958). The α-(1→4) linkage was demonstrated through optical rotation and infrared spectroscopy (Bender et al. 1959). Later, researchers used infrared spectroscopy, methylation reactions, and periodate oxidation to establish that pullulan has both α-(1→4) and α-(1→6) glycosidic bonds in a 2:1 ratio (Shingel 2004, Sowa et al. 1963, Singh & Saini 2012). The structure of pullulan can also be analyzed using the enzyme pullulanase, which specifically cleaves the α-(1→6) glycosidic bonds, leading to complete hydrolysis and the formation of maltotriose units (Hii et al. 2012). Maltotriose consists of three α-(1→4) linked glucopyranose units connected via an α-(1→6) bond, represented as [(1→4)-α-D-Glucopyranosyl-(1→4)-α-D-Glucopyranosyl-(1→6)-α-D-Glucopyranosyl]. Incomplete hydrolysis of pullulan yields other sugars such as panose, maltose, isopanose, and isomaltose (Bender et al. 1959, Sowa et al. 1963). Fig. 1 illustrates the molecular structure of pullulan.

1.5. Microbial Pullulan Producers

Pullulan was initially produced through microbial fermentation using the fungus *Aureobasidium pullulans* for commercial applications. However, not all A. pullulans strains have been characterized for this purpose (Ghimici & Constantin 2020). In addition to Aureobasidium pullulans, other microbial strains can produce pullulan with different ratios of glycosidic bonds, quantities of maltotriose units, and percentages of α-(1→6) linkages within the pullulan framework. A few strains which can produce a pullulan, including *Cytaria darwinii, Cryphonectria parasitica, Aureobasidium mangrovei*, *Cytaria harioti, Tremella mesenterica, Aureobasidium melanogenum, Rhodotorula bacarum, Teloschistes flavicans, Eurotium chevalieri*, and *Rhodosporidium paludigenum*. Table 1 provides

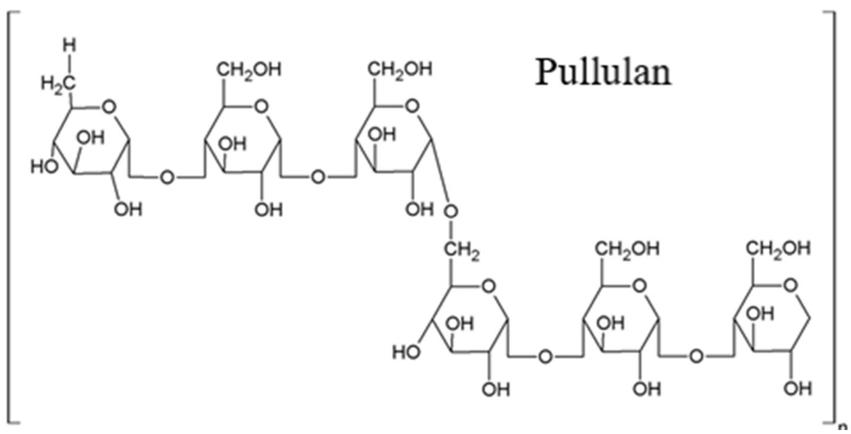

Fig. 1: Molecular structure of pullulan (Ferreira et al. 2015).

Table 1: Fungal sources, structural components, and applications of pullulan.

Sr. No.	Fungal sources	Structural units	Applications	References
1.	*Aureobasidium pullulans*	α-(1→4) linked glucopyranose rings interlinked by α (1→6) linkages.	Biomedical industry, Cosmetics industry, Food industry, Oral care products, etc.	(Al-Araimi et al. 2021), (Basumatary et al. 2022), (Ghimici & Constantin 2020) (Singh et al. 2008)
2.	*Aureobasidium mangrovei*			
3.	*Aureobasidium melanogenum*			
4.	*Cryphonectria parasitica*			
5.	*Cyttaria darwinii*			
6.	*Cyttaria harioti*			
7.	*Eurotium chevalieri*			
8.	*Rhodosporidium paludigenum*			
9.	*Rhodotorula. Bacarum*			
10.	*Teloschistes flavicans*			
11.	*Tremella mesenterica*			

fungal sources, structural components, and applications of pullulan.

1.6. Pullulan Properties

Pullulan typically appears as a fine powder ranging from white to off-white. Microscopic examination reveals that pullulan particles have a smooth surface texture (Li et al. 2018, Soni et al. 2018). Its highly flexible and unique structure contributes to improved hydrodynamic properties (Singh et al. 2008). Pullulan exhibits enhanced solubility and increased solvent resistance. It dissolves rapidly in diluted alkali solutions and in both cold and hot water. However, it is insoluble in organic solvents, except for dimethylformamide and dimethyl sulfoxide. As a dry, non-hygroscopic powder with a moisture content of approximately 10-15%, pullulan does not become sticky. While it can form viscous solutions, it is unable to form gels. The solutions show non-Newtonian, shear-thinning behavior, with shear stress versus shear rate displaying a linear relationship. Tables 2 and 3 present the properties and solubility characteristics of pullulan, respectively.

Table 2: Pullulan properties (Viveka 2023).

Parameters	Specification
Physical state	Powder
Appearance	Whitish or yellowish-white
Flavour	Tasteless and odorless
Toxicity	Non-hazardous and non-cancerous
Molecular weight [kDa]	100 – 250
Consumability	Safe for consumption
Biodegrading nature	Biodegradable
Flow properties	Shear thinning and Non–Newtonian behavior
Polypeptides [%]	Maximum 0.5
Solubility in organic solvents	Insoluble in all organic solvents except formamide and dimethyl sulfoxide
pH of the solution range	5–7
Polydispersity ratio	2.1 – 4.1
Moisture [%]	10 -15
Mineral residue ash [sulfated, %]	Maximum 3
Viscosity range[cP]	80 – 180
Optical activity	Greater than ±160
Range of melting point [°C]	250-350°C

1.7. Gene Expression and Regulation

High pullulan-producing strains, such as *A. melanogenum* ZH27, show upregulation of genes linked to pullulan biosynthesis (UGT1 and Ags2), glycolysis, and by-product synthesis (e.g., trehalose, glycerol, and fructooligosaccharides). This coordinated gene upregulation is associated with increased pullulan yield and enhanced resistance to osmotic stress (Wang et al. 2023).

Transcriptomic analyses of A. pullulans have revealed that biosynthetic genes (upt, pgm, ugp, and pul) are tightly regulated, and that negative regulators such as creA can suppress pullulan synthesis, especially in specific genetic backgrounds (e.g., melanin-deficient mutants) (Chen et al. 2023).

Table 3: Solubility properties of pullulan (Viveka 2023).

Solvent Name	Solubility Conditions
Water	Soluble
DMSO (Dimethyl sulfoxide)	Soluble
Pyridine	Slightly soluble
Ethyl acetate	Insoluble
Acetone	Insoluble
Dimethylformamide (DMF)	Partially soluble
Chloroform	Insoluble

1.8. Morphological and Cellular Changes

Omics-guided studies have linked the presence of swollen cells with large vacuoles to periods of rapid pullulan accumulation, suggesting a physiological adaptation that supports high production levels (Wang et al. 2023).

1.9. Genetic Engineering and Mutagenesis

Targeted genetic modifications, such as knocking out genes involved in melanin synthesis or plasma membrane ATPases (PMAs), have been demonstrated to influence both the amount and molecular weight of pullulan produced. For instance, deleting PMAs in A. pullulans BL06 resulted in a 1.7-fold increase in pullulan yield and a decrease in its molecular weight (Chen et al. 2023). Additionally, UV mutagenesis combined with flow cytometry-based selection has been used to isolate high-yield mutants, as confirmed by omics analyses (Zhang et al. 2024).

1.10. Environmental and Regulatory Factors

Transcriptome analyses conducted under various growth substrates and environmental conditions, such as pH and carbon source, have identified regulatory factors, including pH transcription factors, which significantly enhance pullulan biosynthesis pathways (Wang et al. 2024, Zhang et al. 2023).

1.11. Metabolic Pathways and By-Product Formation

Omics studies have elucidated the balance between pullulan production and byproduct formation. While by-products like trehalose and glycerol assist in osmotic stress management, their accumulation can divert carbon flux away from pullulan, reducing overall yield. Table 4 provides omics insights into pullulan production.

1.12. Status of Pullulan Production in the Commercial Sector

Hayashibara Company Limited in Japan pioneered the commercial production of pullulan in 1976 and secured a patent for its commercialization. It remains a leading producer of food-grade and deionized pullulan, offering products with molecular weights ranging from 100 to 200 kDa (Agrawal et al. 2022). Following the expiry of Hayashibara's patent, several industries began commercializing pullulan.

Hayashibara Co. Ltd. continues to be recognized as the largest supplier of pullulan globally (Coltelli et al. 2020). The brand Listerine, marketed and sold in the US by Hayashibara Company in partnership with Pfizer Inc., is one example of pullulan's applications (Trinetta & Cutter 2025). The company now operates across regions, including North and South America, Europe, Asia, Southeast Asia, and Oceania.

Hayashibara Industries is now known as Nagase Co. Ltd. The demand for pullulan is steadily rising due to its unique properties and potential uses. A recent report, "Pullulan Market Research Report," provides an in-depth overview of market conditions and industrial applications. The global pullulan market is projected to reach USD 89 million by 2028, up from an estimated USD 68 million in 2022. Given its high cost and growing demand worldwide, it is crucial to evaluate the production process of pullulan polymers for industrial applications.

Carbosynth Ltd. in the UK is another prominent industry player that produces pullulan commercially at a significantly lower cost of US $130 per kilogram. Several Indian companies, including Jiyan Chemicals & Pharmaceuticals, Indexim International, Otto Chemie Pvt. Ltd., Kumar Organic Products Ltd., Thermo Fisher Scientific Pvt. Ltd., and Shreeji Pharma International, have also started commercial production of pullulan.

Kumar Organic Products Ltd. is among India's leading pullulan producers, while pharmaceutical-grade pullulan is manufactured locally by Jiyan Chemicals & Pharmaceuticals. In China, multiple companies such as Shanghai Longyu Biotech Co., Ltd., Shandong Hanjiang Chemical Co., Ltd., and Haihang Industry Co., Ltd. have begun producing pullulan on a commercial scale.

The primary Chinese suppliers include Shandong Jinmei Biotechnology Co., Ltd., Shandong Freda Biotechnology Co., Ltd., MeiHua Holdings Group Co., Ltd., Hebei Henbo Bio Technology Co., Ltd., and Shandong Kangnaxin Biotechnology Co., Ltd. Several U.S.-based firms, including Santa Cruz Biotechnology Inc. and MP Biomedicals Inc., have entered the commercial pullulan market. According

Table 4: Omics insights into pullulan production.

Omics approach	Key Findings	References
Transcriptomics	Upregulation of biosynthetic and stress-resistance genes in high-yield strains	(Wang et al. 2023, Chen et al. 2023, 2024)
Functional Genomics	Gene knockouts (e.g., PMAs, melanin) affect yield and polymer properties	(Chen et al. 2023, Zhang et al. 2024)
Morphological Studies	Swollen cells with large vacuoles linked to high pullulan accumulation	(Wang et al. 2023)
Regulatory Networks	pH and carbon source regulation via transcription factors like Appacc	(Wang et al. 2024)

to Fact.MR, the global pullulan market is projected to grow from USD 714.5 million in 2025 to USD 1,163.8 million by 2035, at a compound annual growth rate of 5.0% over the forecast period.

The pharmaceutical industry remains the largest consumer, representing about 40.74% of global demand. The food industry ranks second, with a 36.24% share. Japan leads in consumption, with an annual usage of 667 million tons from 2015 to 2019. China is the second-largest market, holding a 20.65% share, followed closely by the USA at 20.16%. It is widely believed that the future global potential for pullulan usage greatly surpasses current commercial production levels.

1.13. Scope and Original Contribution of the Review

While recent reviews have addressed pullulan production, applications, and waste valorization, they often treat substrate selection, fermentation optimization, strain engineering, and downstream processing as separate topics. This review distinguishes itself with a comprehensive, decision-oriented framework that systematically connects waste substrate properties, fermentation results, key bottlenecks, and omics-based strain improvements. It emphasizes critical barriers such as melanin pigmentation, high broth viscosity, substrate variability, and purification costs, which impact industrial feasibility but are often discussed in isolation elsewhere.

Unlike post-2020 reviews that mainly compile experimental data, this analysis offers a comparative evaluation and future strategies for scale-up. By comparing yields across substrates, fermentation systems, and strains, and integrating techno-economic and biorefinery considerations, it highlights persistent gaps: limited pilot-scale data, scarce cost modeling, and weak integration of omics with process optimization. This focused approach provides a practical roadmap for sustainable, cost-effective pullulan production from agro-waste, surpassing traditional literature reviews.

2. MATERIALS AND METHODS FOR PULLULAN PRODUCTION

A qualitative literature review was selected to thoroughly explore and synthesize the complex information concerning the production of pullulan from agricultural waste using *Aureobasidium pullulans*. The steps undertaken for this review are illustrated in Fig. 2.

2.1. Data Collection and Literature Review

The literature review on pullulan production from agricultural waste using *Aureobasidium pullulans* focused

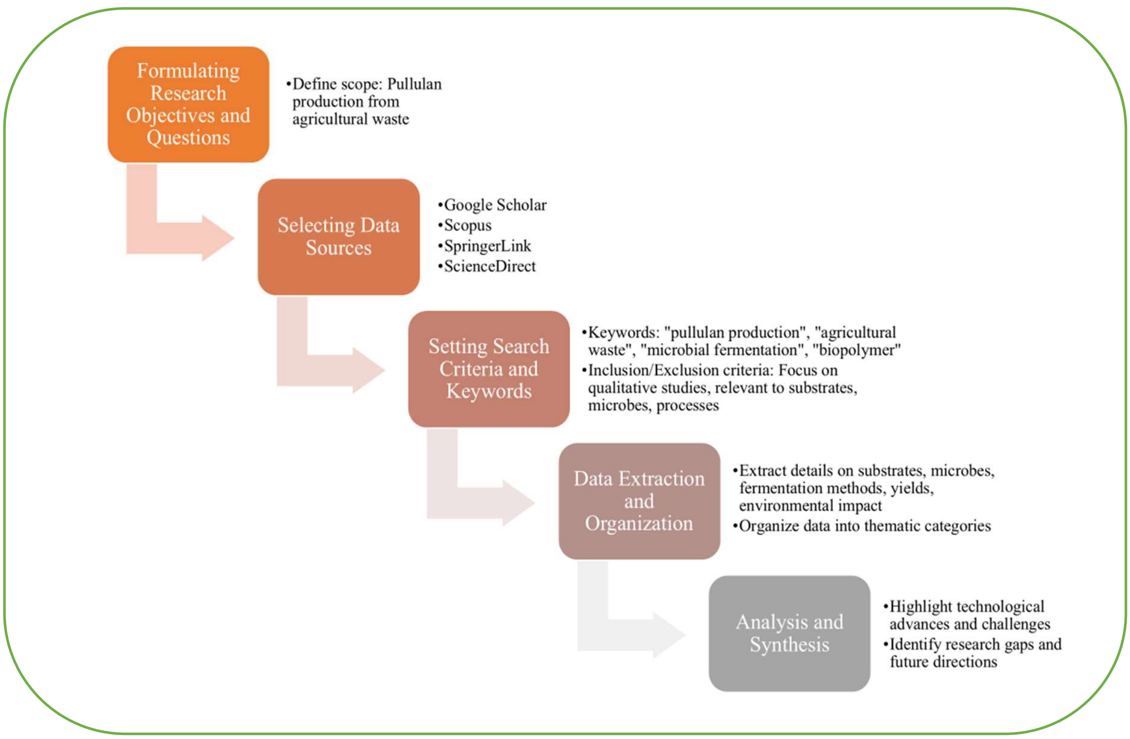

Fig. 2: The steps carried out for the review.

on collecting and synthesizing studies that explored the use of various agro-industrial residues, such as sugarcane molasses, potato starch hydrolysate, citrus peels, and hazelnut husks, as low-cost substrates for fermentation. Data were gathered from reputable scientific databases, including Scopus, Google Scholar, and SpringerLink, using targeted keywords like «pullulan production,» «agricultural waste,» and «*Aureobasidium pullulans*.” Studies were selected based on their relevance to qualitative insights into fermentation processes, substrate optimization, and sustainability.

This review highlights that employing agricultural waste as fermentation media not only decreases production costs but also addresses environmental concerns by valorizing waste streams. Research consistently demonstrates that *A. pullulans* efficiently converts diverse agricultural residues into pullulan through both submerged and solid-state fermentation, with substrate type and composition significantly influencing yield and process efficiency.

2.2. Inclusion and Exclusion Criteria

Studies qualified for inclusion if they-

- Demonstrated pullulan production from agro-industrial or agricultural waste substrates,
- Utilized *Aureobasidium pullulans* or related pullulan-producing microorganisms,
- Provided experimental fermentation data on performance metrics, substrate conversion, yields, or optimization strategies, or
- Addressed the technological, economic, and sustainability implications of waste-based pullulan processes.
- Exclusion criteria encompassed studies that,
- Examined only pure/synthetic substrates without waste valorization relevance,
- Offered inadequate experimental or methodological details,
- Comprised of conference abstracts lacking full-text access, or
- No direct connection to pullulan production.

The literature survey primarily covered studies published between 2000 and 2024, ensuring the inclusion of both foundational and recent research. Title and abstract screening identified relevant studies, followed by a full-text assessment. From approximately 200-300 screened publications, over 100 articles met the criteria for comprehensive qualitative synthesis based on data quality and relevance. Discrepancies across studies were reconciled through comparative analysis, accounting for substrate variations, pretreatment techniques, strain differences, and fermentation parameters, preserving diverse perspectives to reveal critical trends, challenges, and research gaps in this field.

Aureobasidium pullulans

Aureobasidium pullulans, previously identified as *Pullularia pullulans, Hormonema dematioides,* or *Dematium pullulans*, is an imperfect fungus. It is widespread in the natural environment and is commonly found in soil and water, as well as on aged wood and various other plants. Taxonomists have recently categorized *A. pullulans* as an anamorph of *Sydowia polyspora*, a teleomorph (Suzuki et al. 2021). The systematic classification of *A. pullulans* is presented in Table 5.

A. pullulans can consume various carbon sources, such as mannose, glucose, sucrose, maltose, fructose, galactose, xylose, and even agro-industrial waste. The presence of isomerase and hexokinase is necessary for the conversion of the carbon source to UDPG, which is an important precursor for synthesizing pullulan (Sugumaran et al. 2017).

Aureobasidium pullulans has the capacity to produce melanin, so it is often referred to as “black yeast.” Despite being a phytopathogenic fungus, *A. pullulans* does not produce mycotoxins. According to Hermanides-Nijhof (1977), it is a ubiquitous, voracious saprophytic fungus that is frequently isolated from wood, water, soil, and other sources of fungi. A. pullulans has been documented in the phyllosphere, toilets, optical lenses, food and feeds, deteriorating house paints, paper mills, hypersaline waters in salterns, hydrocarbons, synthetic materials, leather, human skin, watercolors, and the surface of decomposing leaves and other plant components. Aureobasidium pullulans was selected for pullulan production because of its high production rate and distinctive qualities. Additionally, this organism generates a variety of enzymes, including β-galactosidase, pectinase, amylase, and xylanase (Viveka 2023).

A. pullulans exhibits five distinct morphologies: chlamydospores, mycelia, swollen blastospores, young blastospores, and yeast-like cells. The morphology of the fungus determines the pullulan yield, although it is unclear

Table 5: Systematic classification of *A. pullulans*.

Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Subclass	Dothideomycetidae
Order	Dothideales
Family	Dothioraceae
Genus	*Aureobasidium*
Species	*Aureobasidium pullulans*

which morphological form of *A. pullulans* is responsible for pullulan production (Oğuzhan 2013). One of the characteristics of this fungus is the formation of dark-colored chlamydospores, and colonies initially appear smooth before becoming covered in a slimy mass. A. pullulans is an industrially significant organism because of its capacity to produce the polysaccharide pullulan.

Under mild conditions, on potato dextrose agar plates at room temperature, *Aureobasidium pullulans* normally grows in 7 days and forms colonies with a diameter of 1-3 cm. The colony is initially smooth, flat, slimy, and leathery, and can be pink, white, yellow, or light brown. The formation of chlamydospores causes the colony to turn brown or black during growth. While mature colonies form a visible, smooth, thin-walled mycelium, young colonies display blastoconidial structures under a microscope. Lobed chains of thick-walled chlamydospores and straight conidia are characteristics of *A. pullulans* that can be used to identify it (Wei et al. 2021).

Genome Sequencing of *Aureobasidium pullulans*

The genomes of four varieties of *Aureobasidium pullulans* have been sequenced, revealing genome sizes between 25.43 and 29.62 Mb, encoding 10,266 to 11,866 predicted proteins (Gostinčar et al. 2014). These genomes contain genes for:

- Degradation of plant material (various enzyme families)
- Numerous sugar transporters
- Potential degradation of plastics and aromatic compounds
- Synthesis of pullulan and siderophores (but not aureobasidin A)
- Stress tolerance (aquaporins, aquaglyceroporins, alkali-metal cation transporters, compatible solute and melanin synthesis, high-osmolarity glycerol pathway, bacteriorhodopsin-like proteins)
- All genomes have a homothallic mating-type locus (Gostinčar et al. 2014).

Proteomics and Extracellular Vesicles (EVs):

Proteomic analysis of EVs from A. pullulans identified 642 proteins, primarily associated with primary metabolism (Černoša et al. 2022). EVs also contained proteins from various biosynthetic pathways, indicating multiple biogenesis routes. Small RNA sequencing of these EVs revealed two potentially novel miRNAs.

Functional Omics Applications:

i. Omics approaches are used to study complex interactions between A. pullulans, fruit hosts, pathogens, and native microflora, especially in the context of postharvest disease biocontrol (Castoria et al. 2001).

ii. Genetic engineering and metabolic pathway analysis (e.g., overexpression of main enzymes in the tricarboxylic acid pathway) have been applied to enhance the production of valuable metabolites (Qin et al. 2024).

Taxonomic Insights: Genomic differences among varieties are substantial enough to suggest reclassification into separate species: *A. pullulans*, *A. melanogenum*, *A. subglaciale*, and *A. namibiae (Gostinčar et al. 2014)*.

The availability of genomic, proteomic, and extracellular vesicle data for *A. pullulans* provides a useful basis for addressing important commercial problems related to pullulan production. The identification of genes involved in melanin synthesis, sugar transport, polysaccharide biosynthesis, and stress tolerance provides direct targets for strain engineering intended to increase substrate utilization efficiency and decrease pigment formation, which significantly increases downstream purification costs. The secretion of metabolic enzymes and regulatory chemicals that may affect polysaccharide export and broth rheology was shown in a proteomic study of extracellular vesicles. This suggests the possibility of modifying the molecular weight and viscosity of pullulan by targeted metabolic regulation.

2.3. Carbonaceous Waste

2.3.1. Potato Starch

Potatoes are cheap and widely accessible agricultural products. Potatoes are mostly composed of starch with traces of sugar. The leftover potato starch from potato crisp production and other potato-processing industries is typically in the form of a homogeneous substrate that is devoid of unnecessary components (Chauhan et al. 2023). Potato-derived starch has been used as an alternative carbon source in several industrial fermentation processes. Some strains of *Aureobasidium pullulans* have enzymes that break down starch, but they are more active against linear α-1,4-glucans than against polysaccharides with α-1,6 linkages. Consequently, starch waste must undergo partial hydrolysis to be considered a viable substrate for pullulan production (Göksungur et al. 2011).

Normally, just before the microbial fermentation process begins, starch should be hydrolyzed to produce sugar. However, using potatoes as the carbon source eliminates the need for the expensive β-amylase enzyme. This is mainly due to the significant amount of highly active β-amylase present in potatoes. In addition, the degree of starch or dextrose equivalent hydrolysis determines the amount of pullulan produced. This shows the overall amount of sugar reduction, expressed as a percentage of glucose. While glucose has a dextrose equivalent of 100, some unhydrolyzed starches have a dextrose equivalent of 0. The pullulan content of the agglutinating substances increased during fermentation using hydrolyzed starch waste as the substrate, and on day six, it reached more than 90% (w/w).

2.3.2. Olive Oil Waste

Phenolic compound-containing waste from olive oil mills is a primary phytotoxic effluent. Olive oil waste damages plants and contaminates the environment (Pinho et al. 2017). Waste from olive oil includes 23 glucose, proteins, and total sugars. These sugars help *Aureobasidium pullulans* grow in the production medium, which increases the pullulan yield. Although a large number of microorganisms cannot survive in the presence of waste olive oil, primarily because of the presence of harmful phenol chemicals. Despite this, *Aureobasidium pullulans* can survive and generate pullulan in the presence of olive oil waste (Youssef et al. 1998).

2.3.3. Sugarcane Bagasse

Sugarcane is one of the main crops grown in large amounts in India. Sugarcane bagasse, a lignocellulosic substance, is produced as solid waste when sugarcane juice is extracted. Approximately 250 kg of bagasse is produced for every ton of sugar produced, for an annual production of 100 million tons. The primary components of sugarcane bagasse are organic matter, lignin, cellulose, hemicelluloses, and ash (Rocha et al. 2015).

2.3.4. Carob Pod

Most crops cannot thrive in the warm and humid climates where carob trees typically flourish. Carob pods can be fed to animals because of their high tannin content. Carob pods are composed of 2% pectin, 5% hemicellulose, 7% cellulose, 20% phenolic components, and 50% sugars. Therefore, carob pod extract has been utilized as a strong carbon substrate in submerged fermentation to produce pullulan (Palaiogianni et al. 2022).

2.3.5. Sweet Potatoes

Sweet potatoes are root vegetables primarily composed of starch and are used for microbial fermentation. Proteins, carbohydrates, lipids, vitamins, and ash are also present in the product. Sweet potatoes are high in starch; however, microbes find it difficult to utilize them. After the saccharification process, hydrolyzed sweet potato can be used as a carbon substrate (Dewan et al. 2013). Fructose, maltose, glucose, maltotriose, and other trace sugars are present in sweet potato hydrolysates.

2.3.6. Brewery Waste

After wort and spent grains are separated, brewing firms release spent grain liquor, a waste product. It contains 12% cellulose, 40% hemicelluloses, 13% lipids, and 3% ash. High biological oxygen demand, which is regarded as a primary contaminant, is also present in spent grain liquor. Its biological oxygen demand harms aquatic life when released into water supplies and pollutes the ecosystem (Chaitanyakumar et al. 2011).

2.3.7. Rice Hull

Rice is widely recognized as a basic essential food in various countries. The outer layer of rice grains, or rice hulls, is produced in large quantities by the rice processing industry. The majority of lignocellulosic materials found in rice hulls are cellulose (50%) and lignin (30%). The composition of rice hulls makes them suitable as carbon substrates for microbial fermentation (Huang & Lo 2019). Microorganisms cannot directly utilize lignocellulosic sugars; therefore, a saccharification procedure is needed to convert this complex into fermentable sugars. After the pretreatment process, rice husk hydrolysate can be utilized as a suitable carbon substrate for pullulan synthesis.

2.3.8. Corn Steep Liquor

Corn steep liquor, a by-product of the maize wet-milling industry, is commonly used as a nutrient source for the production of various microbiological products. This byproduct is primarily used as a nitrogen source. Fungal pigment synthesis can be cultivated using waste stream cellulose generated from corn (Panesar et al. 2015). This waste, which is produced by processing corn and comes from the milling process, is employed as a non-synthetic input in most liquid fertilizer formulations for the cultivation of organic crops. As reported by Chiani et al. (2010), it typically contains 88 g of ash per kg of dry matter, 130–220 g of carbohydrates per kg of dry matter, 205 g of crude protein per kg of dry matter, 525 g of dry matter, and a trace amount of sulfurous acid (<0.01 $g.kg^{-1}$ DM). Additionally, 42% of CSL is a protein (Chiani et al. 2010).

2.3.9. Other Food Industrial Residues

Aureobasidium pullulans has effectively produced pullulan using various food industry residues as substrates. These by-products are rich in carbohydrates and other nutrients, making them suitable and cost-effective alternatives to refined sugars in fermentation. Table 6 shows the conversion of food processing by-products into pullulan by A. *pullulans*.

Although diverse food and agro-industrial wastes have successfully supported pullulan production, the yields varied substantially across substrates and fermentation modes (Table 6). High-performing substrates, such as de-oiled rice bran (54.8 $g.L^{-1}$), potato starch water (54.6 $g.L^{-1}$), sesame seed oil cake (54.5 $g.L^{-1}$), and molasses (45.0 $g.L^{-1}$), delivered superior yields attributable to their abundant fermentable carbohydrates and balanced nutrient

Table 6: Conversion of food processing by-products into pullulan using *Aureobasidium pullulans*.

Processing food waste	Fermentation process	Fermentation time, temperature, etc.	Pullulan yield [g.L^{-1}]	References
Molasses	Shake and flask (Submerged fermentation)	5 days, 35°C, 150 rpm,	45.00	(Srikanth et al. 2014)
Beet Molasses	Stirred tank bioreactor	5 days, 400 rpm, 28°C, aeration rate 2 vvm	6.6	(Göksungur et al. 2004)
	Shake flasks for fermentation.		16.7	
Cassava bagasse	Solid-state fermentation	5 days, 30°C	19.00	(Sugumaran et al. 2014)
De-oiled rice bran	Shake and flask (Submerged)	7 days, 30°C, 150 rpm	54.8	(Singh & Kaur 2019)
Potato starch water	Stirred tank reactor fermentation (Submerged)	5 days,28°C, 500 rpm	54.57	(An et al. 2017)
Rice hull	Stirred tank reactor fermentation (Submerged)	3 days, 28°C, 400 rpm, aeration rate 3 L.min^{-1}	22.20	(Wang et al. 2014)
Sesame seed oil cake	Solid-state in a flask	25°C, 2 h, 200 rpm	54.50	(Mirzaee et al. 2020)

profiles, requiring minimal pretreatment. Conversely, beet molasses produced markedly lower yields (6.6-16.7 g.L^{-1}) in bioreactors, likely due to its sucrose-dominant composition, inhibitory compounds, and shear stress at high agitation rates (400-500 rpm), which impair exopolysaccharide biosynthesis by *Aureobasidium pullulans*.

Furthermore, fermentation mode played a critical role, with solid-state fermentation (SSF) achieving superior yields (19.0-54.5 g.L^{-1}) relative to submerged fermentation (SmF, 6.6-54.8 g.L^{-1}) despite simpler process control. Sesame seed oil cake excelled in SSF because of its concentrated nutrient content and reduced dilution effects, whereas variations in fermentation time, temperature, and agitation influenced oxygen transfer, metabolic activity, and broth viscosity. These observations highlight that pullulan production efficiency is governed by the interplay of substrate composition, fermentation strategy, and process parameters, rather than by substrate type alone.

2.4. Hydrolysis of Waste

Globally, both rural and urban areas in many developing countries, including India, face significant challenges in solid waste management. In some cases, bioconversion of these wastes, such as the microbial synthesis of different enzymes, organic acids, and feed, can be both economically advantageous and reduce environmental pollution (Rishi et al. 2020).

Agro-based industries produce a vast quantity of waste, and if the waste is discarded untreated, it can result in significant environmental issues. However, these agro-industrial wastes are rich sources of nutrients and organic and inorganic matter. This waste can be used as an alternative carbon or nitrogen source for producing various microbial products. It helps reduce the environmental pollution generated by the direct removal of untreated waste and is also economically beneficial (Singh et al. 2021).

Commercially relevant glucose yields can only be obtained from the hydrolysis of cellulose in the presence of a catalyst. (Hu et al. 2015). There are three basic types of catalysts: enzymatic, concentrated acid, and diluted acid. The key benefits of employing enzymatic catalysts are their high specificity, lack of by-products, ability to function in mild environments, environmental friendliness, and the fact that a tiny amount of enzyme produces large yields. However, to use enzymatic hydrolysis, pretreatment is required to free up the structure and provide enzyme access to the active sites. Pre-treatment is frequently carried out using physical methods that require a lot of energy, high pressure, and temperature, or the application of a chemical solvent, such as a diluted acid. Additionally, lignin may block enzyme hydrolysis. Long reaction periods, large reactors, and high enzyme costs are additional drawbacks of enzymatic hydrolysis (Orozco et al. 2007).

The only pressure involved in concentrated acid operations is that produced when materials are pumped from one vessel to another at comparatively low temperatures. The breakdown of sugars into unwanted byproducts is minimized by low pressures and temperatures. The hydrogen bonding in the cellulose chain is broken by concentrated acid, leaving it in an amorphous condition. After decrystallization, cellulose and acid combine to form a homogenous gel that permits hydrolysis reactions. The solution can then be diluted by adding water at low temperatures, which creates an ideal environment for glucose development (Orozco et al. 2007).

Dilute acid hydrolysis is usually carried out at a higher temperature range of 200–240°C, while a lower temperature is required for concentrated acid hydrolysis and higher glucose yields of approximately 90% are achieved using 30–70% sulfuric acid (Niju et al. 2020). However, concentrated acid hydrolysis requires a large amount of acid, which causes corrosion to equipment, and the main drawback of concentrated acid is the high cost of acid, a recovery step is necessary to lower the cost.

Despite its unique features and potential applications, pullulan has limited industrial use due to its high production cost. Pullulan production requires a cost-effective strategy in order to increase its appeal for industrial uses. Several strategies have been employed to lower the price of producing pullulan, such as enhanced strains, genetic engineering advancements, and the discovery of low-cost sources of carbon and nitrogen. Thus, inexpensive substrates are crucial for developing processes that are both profitable and practical.

Mishra et al. (2018) compared pullulan production to that of xanthan gum. They discovered that the cost of pullulan production was three times higher than that of xanthan gum. The production of pullulan from raw material utilization accounts for approximately 30% of the total production costs. Pullulan's unique structural properties probably necessitate a complex and expensive purification process, which accounts for the majority of its higher cost. To lower expenses and improve pullulan's economic feasibility for industrial applications, this study emphasizes the necessity for additional optimization of pullulan production techniques. The price difference between xanthan gum and pullulan may be attributed to economies of scale and decreased demand. The authors stress how crucial it is to deal with these financial aspects to encourage widespread adoption (Mishra et al. 2018).

The type of fermentation process, fed-batch or continuous, also has a significant impact on the amount of pullulan generated by *Aureobasidium pullulans*. Pullulan production is also significantly affected by fermentation conditions. The components of the fermentation medium are used by *Aureobasidium pullulans* cells during fermentation to produce pullulan and cellular biomass. The rheological response of the fermentation broth is influenced by cellular biomass, cell debris, and pullulan accumulation during fermentation. During pullulan production, the rheology of the fermented broth changes from Newtonian to non-Newtonian. Increased viscosity in the fermentation broth reduces the ability of microbial cells to use oxygen and growth factors, leading to decreased pullulan productivity during fermentation. Controlling various parameters during fermentation is necessary (Viveka 2023).

Acid and enzymatic hydrolysis are common methods for breaking down agro-waste into sugars for pullulan production; however, their cost-effectiveness varies in this context. Acid hydrolysis is cheaper and faster for sugar release; however, it produces inhibitors such as furfurals and acids that harm *Aureobasidium pullulans* growth and pullulan yield, raising detoxification costs. Enzymatic hydrolysis provides better sugar quality, fewer inhibitors, and reliable fermentation, leading to a consistent pullulan output despite higher enzyme costs and slower times. For pullulan, enzymatic methods are preferable for process stability and product purity, whereas acid hydrolysis is suitable for setups with detoxification or multi-product recovery. Few studies have compared these economically for pullulan, indicating the need for industrial-scale assessments.

2.5. Pullulan Production Through Fermentation by Using Hydrolysed Cellulosic Waste

A fungus called *Aureobasidium pullulans* produces a commercially available carbohydrate polymer, pullulan. *Aureobasidium pullulans* cells produce pullulan as an exopolysaccharide in the late stages of their life cycle, and it builds up as slime on the cell surface. Various strains of A. pullulans have been documented to produce pullulan via fermentation. (Cheng et al. 2011a). For every strain of *Aureobasidium pullulans* to produce pullulan, a particular media composition and environment are required. The production of pullulan is greatly dependent on the composition of the fermentation medium, specifically the nitrogen source, carbon source, pH, and medium supplements of the fermentation medium. The type of fermentation system, batch, fed-batch, or continuous, also significantly affects the amount of pullulan produced by *A. pullulans* (Youssef et al. 1999).

Pullulan production is significantly influenced by fermentation conditions. During the fermentation process, *Aureobasidium pullulans* metabolizes the components of the medium to form biomass and secretes pullulan. The medium changes from Newtonian to non-Newtonian owing to the effects of cellular biomass, cell detritus, and accumulated pullulan during fermentation on the rheology of the fermentation broth. Because of the increased viscosity of the fermentation medium, pullulan production is less efficient because microbial cells are unable to use oxygen and growth nutrients effectively. The production of pullulan by *Aureobasidium pullulans* can be increased by better controlling different aspects of the fermentation process (Cheng et al. 2011b). Fig. 3 illustrates the pullulan synthesis mechanism.

2.6. Factors Affecting the Production of Pullulan

The production of pullulan is influenced by several variables, including inoculum size, age, temperature during fermentation, and medium pH. *A. pullulans* consumes the medium ingredients during fermentation to produce pullulan and cellular biomass. By altering the rheology of the fermentation broth, biomass, and cell debris buildup, they also have an impact on pullulan production (Wani

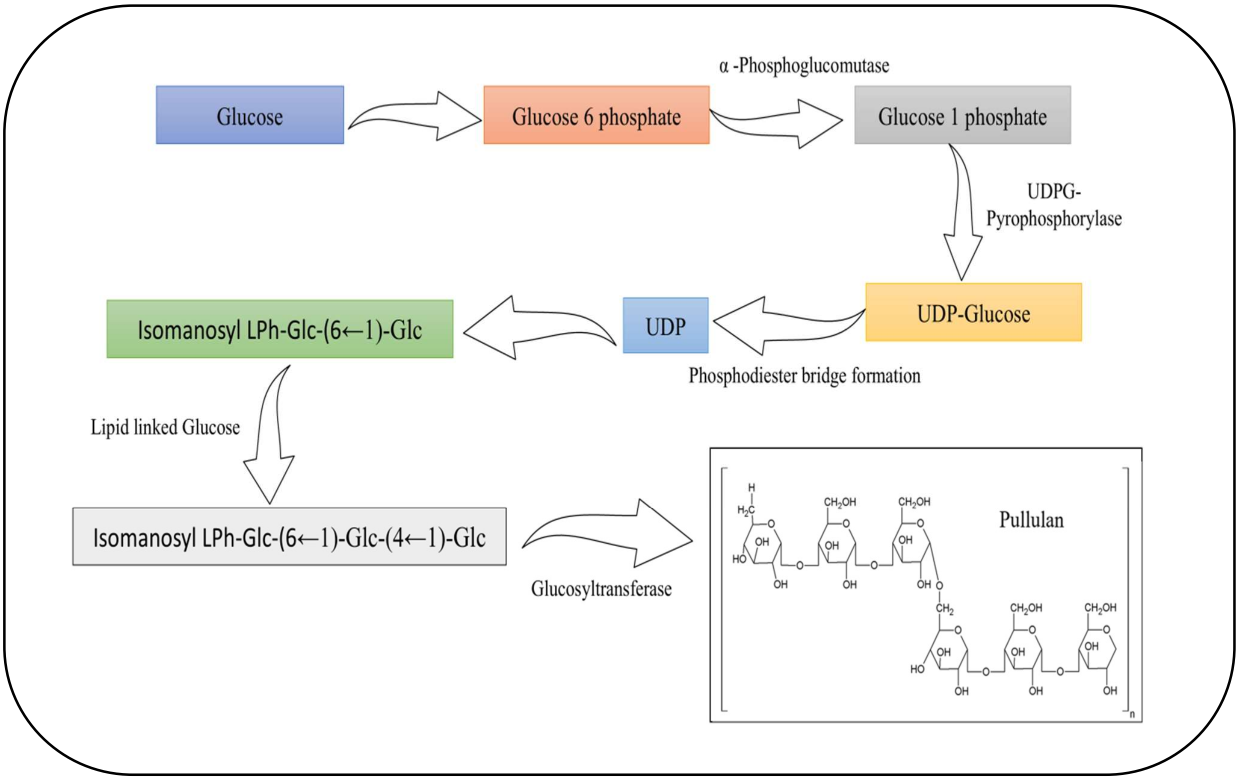

Fig. 3: Pullulan synthesis mechanism. (Mishra et al. 2023).

et al. 2021). A higher viscosity in the fermentation broth reduces the quantity of oxygen and medium components that *A. pullulans* can consume, thereby slowing down pullulan production. The production of pullulan in shake flasks is greatly affected by the composition of the fermentation medium, including the nitrogen source, carbon source, medium supplements, and medium pH. Pullulan production is affected by the following parameters, as demonstrated:

2.6.1. Temperature

Fermentation temperature is a significant factor in pullulan production. The optimum temperature for the growth of microbial cells ranges from 25–30°C, which initiates pullulan production during shake flask fermentation. Lower temperatures slow cell growth and pullulan yield, whereas higher temperatures reduce pullulan yield. The ideal temperature range for pullulan production is between 28 and 30°C (Wu et al. 2012).

2.6.2. pH

One crucial element of the fermentation process is the pH of the fermentation medium. The pH of the strain changes according to the type of microbe used. The parameters of the process and the formulation of the fermentation medium affect the pH of the medium. Variations in pH have a significant effect on the morphological features of the cell culture in the fermentation broth, which slows down the fermentation process and results in less pullulan production. According to most studies, a pH range of 5.0–7.6 is ideal for the growth of microbial cells and pullulan production (Gaur et al. 2010).

2.6.3. Time

Another crucial factor in pullulan synthesis is the fermentation period, which is determined by the requirements of the microbial isolate. After being injected into the fermentation broth, the cultured cells adjust to their surroundings and initiate fermentation. Pullulan production and growth of cells take place during the log phase of cells. The cells enter the stationary phase as fermentation continues, inhibiting pullulan production. Temperature and medium composition affect the duration of pullulan production. Each strain had a different fermentation period. Under shake-flask fermentation, three to seven days is the optimum fermentation period for pullulan production (Hamidi et al. 2019).

2.6.4. Inoculum Size

The term “inoculum size” refers to the number of microbial cells required to inoculate the fermentation medium to produce pullulan. When sufficient microbial cells are present, *A. pullulans* cells can grow to the necessary size for pullulan production. The pullulan yield and cell biomass increased as the inoculum size increased. The reduced yield of pullulan during fermentation is caused by insufficient cellular biomass growth from smaller microbial inoculum sizes (Mirzaee et al. 2020). According to reports, the optimal inoculum size of *Aureobasidium pullulans* for pullulan production during shake-flask fermentation is 5% (Ding et al. 2020).

2.6.5. Inoculum Age

One of the crucial factors influencing pullulan production from *Aureobasidium pullulans* is the age of the inoculum. The most active form of *Aureobasidium pullulans* is yeast-like cells, which are normally used for inoculation (Jiang et al. 2018). When producing pullulan, microbial cells in the log phase are typically preferred for inoculation over those in the lag phase. Fermentation times are shortened when the cells of *Aureobasidium pullulans* are in the log phase. However, inoculating *Aureobasidium pullulans* cells during the stationary phase lengthens the fermentation process and decreases the growth uptake factors required to produce pullulan. According to published research, the best inoculum age for pullulan production is one to four days old (Haghighatpanah et al. 2021).

2.6.6. Agitation

Agitation is important for the pullulan production process during shake flask fermentation. The viscosity of the fermentation medium often increases during pullulan formation, making it difficult for the microbes to use the medium's ingredients (Kumar et al. 2019). During fermentation, agitation keeps the medium homogeneous and helps in the constant mixing of its constituent parts. In a homogeneous medium, microorganisms have constant access to oxygen (Lazaridou et al. 2002) and easy access to the nutrients of the medium (Jafari et al. 2007). It promotes pullulan accumulation and proliferation of microbial cells (Jafari et al. 2007, Lazaridou et al. 2002). A concentration gradient between the inside and outside of the cell, as well as constant mass transfer across the cell wall, is maintained by appropriate agitation (Choudhury et al. 2012). The type of microbe also determines whether agitation is necessary.

2.6.7. Carbon Sources

The primary source of energy in the fermentation medium is the supply of carbon, which promotes microbial cell growth. There have been reports on the production of pullulan using a variety of carbon sources, including lactose, sucrose, galactose, glycerol, glucose, xylose, arabinose, cellobiose, rhamnose, inulin, fructose, and maltose (Singh et al. 2008). The majority of research work has shown that when sucrose is utilized in shake-flask fermentations as a carbon source, the maximum pullulan production occurs (Badhwar et al. 2018, Badhwar et al. 2019, Chen et al. 2018, Chen et al. 2020, Chen et al. 2020b, Haghighatpanah et al. 2021, Hamidi et al. 2019, Jiang 2010, Jiang et al. 2019). During fermentation, sucrose is the most effective inducer of β-fructofuranosidase in *Aureobasidium pullulans* cells because pullulan is a nonreducing sugar. When the sucrose concentration in the fermentation medium is low, β-fructofuranosidase catalyzes the conversion of sucrose into glucose and fructose.

However, in the initial stages of fermentation, fructose and sucrose are bound to each other. β-fructofuranosidase also promotes the accumulation of ketose when sucrose concentration is high (Sheng et al. 2016). In shake-flask fermentations, glucose is another common carbon source in the fermentation medium, which is used to produce pullulan (Cheng et al. 2011a, Gaur et al. 2010, Hamidi et al. 2019, Liu et al. 2018, Ma et al. 2014, Prasongsuk et al. 2005, 2006). The Embden-Meyerhof-Parnas pathway is used in A. *pullulans* biosynthesis to produce pullulan from glucose during fermentation (Fernandes et al. 2024). Less pullulan yield from glucose has been found in most investigations than that from sucrose (Sheng et al. 2016).

2.6.8. Nitrogen Sources

During fermentation, the nitrogen source controls the metabolic activity of the cells and affects pullulan production (Wang et al. 2015). Pullulan production has been reported using a variety of complex organic nitrogen sources, such as urea, peptone, yeast extract, and ammonium sulfate, as well as inorganic nitrogen sources, such as ammonium oxalate, ammonium formate, ammonium chloride, ammonium citrate, ammonium nitrate, ammonium acetate, copper sulfate, and sodium nitrate. In most shake-flask fermentations, a combination of yeast extract and ammonium sulfate has been identified as the optimal nitrogen source for *Aureobasidium pullulans* to produce pullulan. Pullulan has also been manufactured using only yeast extract (An et al. 2019, Hamidi et al. 2019, Jiang 2010). This is an extremely multifaceted organic nitrogen supply composed of many peptides, amino acids, vitamins, minerals, and other substances.

2.7. Downstream Processing of Pullulan

Downstream processing is a multi-step procedure used to safely recover a microbial product while maintaining its biological activity and purity. It must be effective, reproducible, and maximize product recovery yield while requiring the least number of resources to produce (Singh 2014). Selecting inexpensive or complex substrates to formulate fermentation media on an industrial scale typically results in lower upstream-processing costs. However, the downstream process requires more steps to separate inexpensive/complex substrates, which are highly impurity-filled, thereby increasing the cost of the final product. However, using highly purified substrates in the fermentation medium reduces the complexity of downstream processing steps, but their cost is high.

Before downstream processing, the fermented medium may occasionally be pretreated to preserve the microbial product and prevent its degradation (Svarovsky 2001). To fulfill the requirements for a certain microbial product's usage, various downstream processing methods are used to achieve the required level of purity. In general, it is best to reduce the steps involved in downstream processing to reduce the loss of microbiological products at various purification steps, shorten process times, and lower production costs.

Downstream processing of pullulan includes liquid-liquid separation after solid-liquid separation. Conventional techniques for downstream processing of pullulan include precipitation using organic solvents, dialysis, ultrafiltration, and gel filtration chromatography. NMR spectroscopy, chromatographic techniques, and FTIR spectroscopy are further used to confirm the pullulan structure (Shingel 2002). The design of pullulan production from agricultural waste is shown in Fig. 4.

3. PROBLEMS FACED DURING PULLULAN PRODUCTION

Despite its unique properties, the high production cost of pullulan, which is often three times higher than that of other polysaccharides such as xanthan or dextran, remains a critical

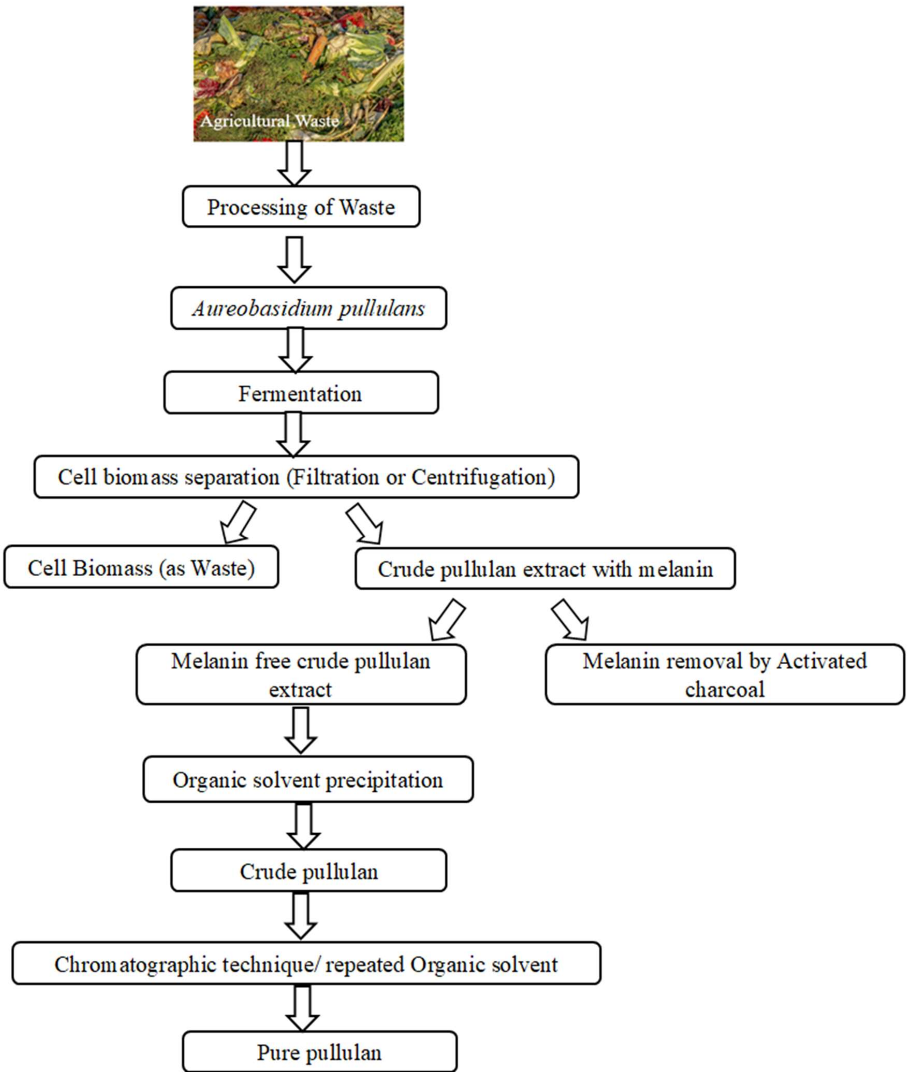

Fig. 4: Design of pullulan production from agricultural waste (Singh et al. 2019).

barrier to its widespread industrial adoption. This is due to several factors.

- High Cost and Complexity of Raw Material Preparation:

  The high cost of nutrients and raw materials, even when using waste substrates, and the need for extensive pretreatment and hydrolysis of some residues (Cruz-Santos et al. 2023). This captures the dual challenge of expensive nutrients and the need for extensive pretreatment or hydrolysis when using certain agricultural or food industry residues as substrates for pullulan production. The cost of raw materials can account for up to 30% of the total production costs, and the requirement for additional processing steps further increases overall expenses and operational complexity (Mehta et al. 2014).

- Downstream Processing:

  Downstream processing challenges, such as the high viscosity of fermentation broths and the co-production of melanin pigments, complicate purification and increase costs.

i. High viscosity of the culture broth:

The high viscosity of the broth is a major problem faced during pullulan production. During fermentation, pullulan may degrade (pullulanolysis) due to the production of melanin. In the fermentation medium, microbial cells, residual media components, cellular debris, and extracellular metabolites are left after the fermentation process is completed. Hence, for the separation of these impurities, an alternative downstream process is needed before the precipitation of pullulan (Ray & Moorthy 2007).

ii. Melanin production:

The pentaketide pathway is involved in the intracellular and extracellular synthesis of melanin pigments and pullulan. Medium composition and culture conditions affect melanin synthesis. Activated charcoal has been used to separate melanin (Kachhawa et al. 2003). However, the main disadvantage of activated charcoal is that some pullulan and fine charcoal powders are lost due to contamination. The viscosity of the broth also increased with the introduction of activated charcoal. Therefore, it is necessary to develop another technique for separating the pigments in melanin. The need for colorless or low-melanin-producing strains is pressing, as melanin removal adds a costly step to the process.

4. INDUSTRIAL AND COMMERCIAL APPLICATIONS OF PULLULAN

Pullulan has several uses in the biomedical industries, food, cosmetics, and pharmaceutical industries because of its special qualities (De Souza et al. 2023). Pullulan finds application in the pharmaceutical business as a packaging material. According to Tabasum et al. (2018), pullulan can also be used as a low-calorie polysaccharide in food packaging, dietary fibers, drinks, stabilizers, binders, and coating materials. Pullulan serves as a novel biomaterial in tissue engineering and various biomedical fields, and its derivatives have applications in gene targeting and medication (Tabasum et al. 2018).

Pullulan has various uses in different kinds of cosmetic products, such as cosmetic lotions, cosmetic powders, rouges, cosmetics for use around the eyes, facial packs, shampoos, set lotions, hair lacquers, and toothpaste (Roy et al. 2020). The exclusive characteristics and physical features of pullulan make it an ideal biopolymer for use in the cosmetics industry. The greatest application of this polysaccharide is in oral-care products. These products using pullulan have been broadly commercialized (Singh & Saini 2012). Owing to its non-irritating properties, pullulan is widely used in a range of cosmetic products, powders, hair styling products, lotions, shampoos, and facial masks. It is used in mascara and eyelash products as a film-forming agent to increase lash length and volume, resulting in a more voluminous and defined appearance. Additionally, pullulan is employed in hair care items such as gels, hair sprays, and styling creams, where it acts as a film-forming and fixative agent, providing hold and improving the durability of hairstyles (Coltelli et al. 2020).

Pullulan-based skin care products are useful as a skin-whitening agent. It supports the tissue and corrects the structural architecture, which is necessary for tissue regeneration (Xiao et al. 2012). Pullulan is incorporated into anti-aging formulations, including firming lotions and wrinkle creams. It offers a temporary tightening effect, providing the skin with a smoother and more youthful appearance (Chen et al. 2014).

Pullulan can be utilized in next-generation point-of-care testing for the storage of labile biomolecules as stable water-soluble pellets for a long time, and drug capsules made of pullulan are welcomed by vegetarians, Muslims, diabetics, and patients with restricted diets (Liu et al. 2017).

Cell division and growth mechanisms for skin renewal slow down as the skin ages (Coltelli et al. 2020). A novel cosmetic formulation may incorporate an efficient delivery system capable of crossing the skin barrier and releasing active compounds in the targeted skin layers. When an element is delivered to a certain layer, the intercellular connection is restored, and the aging or damaged dermis structure is hydrated and renewed (Prausnitz et al. 2004).

Table 7: Properties of pullulan and their cosmeceutical properties (Coltelli et al. 2020).

Pullulan properties	Cosmetic application
White to off-white appearance, non-toxic, odourless, water-soluble, adhesive, and tacky	Used as an ingredient in liquid and paste rouges to improve texture and adhesion
Film-forming, safe, and non-irritant	Ingredients in eyeliners and eye shadows for smooth application and skin compatibility
Moisturizing, adhesive, film-forming, retains continuity during peeling, with film shrinkage providing skin surface tension	Active component in facial packs to enhance skin feel and performance
Foam-enhancing and stabilizing properties	Used in hair shampoos to improve foam quality and stability
High coherence, viscosity, non-toxic, and foam stability during storage	Toothpaste ingredient for texture and foam consistency
Good water solubility contributes to transparent film formation	Applied to face masks and hair styling products for aesthetic and functional benefits
Strong film-forming ability, adhesive, hair-setting capacity, and ease of removal	Used in body lotions and hair lacquers for styling ease and product removal

Traditionally, the carriers for cosmetic products have been polymers with strong water solubility, such as carboxymethyl cellulose, alginate, hydroxyethyl cellulose, starch, and methyl cellulose. Novel cosmetic products can also be developed using natural polysaccharides as carriers (Danti et al. 2019, Morganti & Coltelli 2019). According to Parul and Dubey (2018), it is completely non-mutagenic, non-immunogenic, and non-carcinogenic. It is highly water-soluble, tacky, and low-viscosity, with a high moisture absorption rate. The comparatively low viscosity of pullulan makes it an excellent binder for cosmetic formulations (Parul & Dubey 2018).

Pullulan is an element that can increase the quality of cosmetic products. Pullulan can be used in any amount in a cosmetic product without limitations due to its GRAS designation and lack of toxicity. Pullulan's photoprotective qualities have led to a rise in the demand for hair and skin care products rather than makeup with chemicals. Table 7 shows the properties of pullulan and its cosmeceutical properties.

5. CONCLUSIONS

Agro-industrial waste presents a sustainable and cost-effective carbon source for producing pullulan, thanks to its abundance, nutritional content, and potential for waste valorization. Although extensive research has demonstrated the feasibility of synthesizing pullulan from various waste feedstocks, large-scale industrial application is still limited by high downstream processing costs. These costs are primarily driven by melanin pigmentation, excessive broth viscosity, and inconsistent purity when using waste substrates, all of which increase purification requirements and negatively impact economic viability. Among various mitigation approaches, omics-informed strain engineering combined with precise fermentation control has proven to be the most promising solution. Insights from genomics, transcriptomics, and proteomics have led to the development of *Aureobasidium pullulans* strains that exhibit reduced pigmentation, improved waste substrate utilization, and controlled polysaccharide molecular weight. These advances, alongside process optimization, aim to deliver higher yields and greater consistency. Nonetheless, downstream technologies alone are insufficient without concurrent upstream biological improvements. Key gaps in the literature include the lack of pilot-scale demonstrations, waste-specific techno-economic assessments, and integrated strain-process strategies. Addressing these gaps through multidisciplinary efforts is essential to transitioning pullulan production from laboratory prototypes to competitive, circular bioeconomy processes.

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